

amino group of the adjacent lysine residue,  $[\epsilon\text{-}\gamma]\text{-Glu}$ ,  $[\epsilon\text{-}\gamma]\text{-Glu-}[\alpha\text{-}\gamma]\text{-(Glu)}_{1,3}$ ,  $[\epsilon\text{-}\alpha]\text{-(Phe)}_{1,3}$ ,  $[\epsilon\text{-}\alpha]\text{-(Tyr)}_{1,3}$ ,  $[\epsilon\text{-}\alpha]\text{-(Trp)}_{1,3}$ ,  $[\epsilon\text{-}\alpha]\text{-(Lys)}_{1,3}$  and  $[\epsilon\text{-}\alpha]\text{-(Arg)}_{1,3}$ , wherein  $[\epsilon\text{-}\gamma]$  represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate,  $[\alpha\text{-}\gamma]$  represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate,  $[\epsilon\text{-}\alpha]$  represents a peptide bond between the epsilon amino acid of lysine and the alpha carboxyl group of the indicated amino acid and the subscript indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds.

Please delete paragraph [0024], on pages 8 & 9, and replace it with the following paragraph:

**[0024]** According to a preferred embodiment of the invention, the cytolytic peptide is melittin or an analog or derivative thereof. Melittin is isolated from bee venom and is a 26 amino acid amphiphilic alpha-helix (Blondelle et al., (1991) Biochemistry 30: 4671-4678; Dempsey et al., (1991) FEBS Lett. 281: 240-244.). The amino acid sequence of melittin is shown in Table 1. Residues 1-20 are predominantly hydrophobic and residues 21 to 26 are hydrophilic and basic. Melittin has antibiotic activity, but in mammals it is lytic for leukocytes, red blood cells and a wide variety of other cells. Compounds similar to melittin, which are also within the scope of the invention, include bombolitin from bumblebee venom (17 amino acid amphiphilic alpha-helix), mastoparan from wasp venom (14 amino acid amphiphilic alpha-helix) and crabrolin from hornet venom (13 amino acid amphiphilic alpha-helix) (Argiolas, A. and Pisano, J. J., 1985, J. Biol. Chem. 260, 1437-1444.).

TABLE 1 Amino Acid Sequence of Selected Cytolytic Peptides

**Amoebapore Helix 3 (*Entamoeba histolytica*)**

NH<sub>2</sub>-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys-CONH<sub>2</sub> (SEQ ID NO: 3)

**Cecropin A (*Antheria pernyi*)**

NH<sub>2</sub>-Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ile-Glu-Lys-Val-Gly-Gln-Asn-Ile-Arg-Asp-Gly-Ile-Ile-Lys-Ala-Gly-Pro-Ala-Val-Ala-Val-Val-Gly-Gln-Ala-Thr-Gln-Ile-Ala-Lys-COOH (SEQ ID NO: 4)

**Cecropin B (*Antheria pernyi*)**

NH<sub>2</sub>-Lys-Trp-Lys-Ile-Phe-Lys-Lys-Ile-Glu-Lys-Val-Gly-Arg-Asn-Ile-Arg-Asn-Gly-Ile-Ile-Lys-Ala-Gly-Pro-Ala-Val-Ala-Val-Leu-Gly-Glu-Ala-Lys-Ala-Leu-COOH (SEQ ID NO: 5)

**Cecropin D (*Antheria pernyi*)**

NH<sub>2</sub>-Trp-Asn-Pro-Phe-Lys-Glu-Leu-Glu-Lys-Val-Gly-Gln-Arg-Val-Arg-Asp-Ala-Val-Ile-Ser-Ala-Gly-Pro-Ala-Val-Ala-Thr-Val-Ala-Gln-Ala-Thr-Ala-Leu-Ala-Lys-COOH (SEQ ID NO: 6)

**Melittin (*Apis mellifera*)**

NH<sub>2</sub>-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-COOH (SEQ ID NO: 7)

Please delete paragraph [0034], on page 13, and replace it with the following paragraph:

**[0034]** A particularly preferred cytotoxin is an amoebapore derivative: NH<sub>2</sub>-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys-CONH<sub>2</sub> (SEQ ID NO: 3).

Please delete paragraph [0040], on page 15, and replace it with the following paragraph:

**[0040]** Particularly preferred procytotoxins include amoebapore, its analogs and its derivatives that contains one or more  $\gamma$ -linked glutamate residues linked via a peptide bond to the epsilon amino group of at least one lysine, preferably the C-terminal-most lysine (hereinafter " $\gamma$ -glutamate-masked amoebapore analog"). A particularly preferred procytotoxin has the following structure: Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-

Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys- $[\epsilon-\gamma]$ -Glu- $[\alpha-\gamma]$ -(Glu) (SEQ ID NO: 8), wherein  $[\epsilon-\gamma]$  represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate and  $[\alpha-\gamma]$  represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate.

Please delete paragraph [0041], on page 15, and replace it with the following paragraph:

**[0041]** In addition, amoebopore and other cytotoxic peptides can be modified with other amino acids. One such exemplary protoxin has the following structure: Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys- $[\epsilon-\alpha]$ -Phe (SEQ ID NO: 9), wherein  $[\epsilon-\alpha]$  represents a peptide bond between the epsilon amino group of lysine and the alpha carboxyl group of the adjacent phenylalanine. Another exemplary protoxin that can be activated by chymotrypsin-like activity has the following structure: Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys- $[(\epsilon-\alpha)]$ -Phe)-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys- $[\epsilon-\alpha]$ -Phe (SEQ ID NO: 10), using the same nomenclature and where Lys  $[(\epsilon-\alpha)]$ -Phe)-Leu represents a linkage between the epsilon amino group of lysine and the alpha carboxy group of phenylalanine, and a standard peptide linkage between lysine and phenylalanine. Of course, the phenylalanine may be replaced with other amino acids, such as tyrosine and tryptophan in the case of chymotrypsin-like activity. In some instances, in order to invoke trypsin-like activity, it may be beneficial to utilize positively charged amino acids, like arginine and lysine, instead of phenylalanine.

Please delete paragraph [0042], on page 16, and replace it with the following paragraph:

**[0042]** Other particularly preferred procytotoxins include melittin, its analogs and its derivatives that contain at least one  $\gamma$ -linked glutamate residue linked via a peptide bond to the epsilon amino group of a lysine (hereinafter " $\gamma$ -glutamate-masked melittin analog"). As indicated in Table 1, melittin has two lysines and two adjacent arginines near its C-terminus. When one of the lysines is so masked, it is expected that the free alpha carboxyl group would act to neutralize the adjacent arginine, further

contributing to the inhibition of the toxic activity of melittin. A particularly preferred procytotoxin has the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys([ε-γ]-Glu)-Arg-Lys([ε-γ]-Glu)-Arg-Gln-Gln (SEQ ID NO: 11), wherein -Lys-([ε-γ]-Glu)-Arg- represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate and a standard peptide bond between the lysine and arginine residues. Of course, -Lys-([ε-γ]-Glu)-Arg- can be replaced, for example, by -Lys([ε-α]-Phe)-Leu-, as detailed above, and phenylalanine can be replaced by other amino acids like tyrosine and tryptophan to invoke chymotrypsin-like activity. In some instances, when trypsin-like activity is being invoked, it may be beneficial to utilize positively charged amino acids, like arginine and lysine, instead of phenylalanine in this latter example.

Please delete paragraph [0044], on page 17, and replace it with the following paragraph:

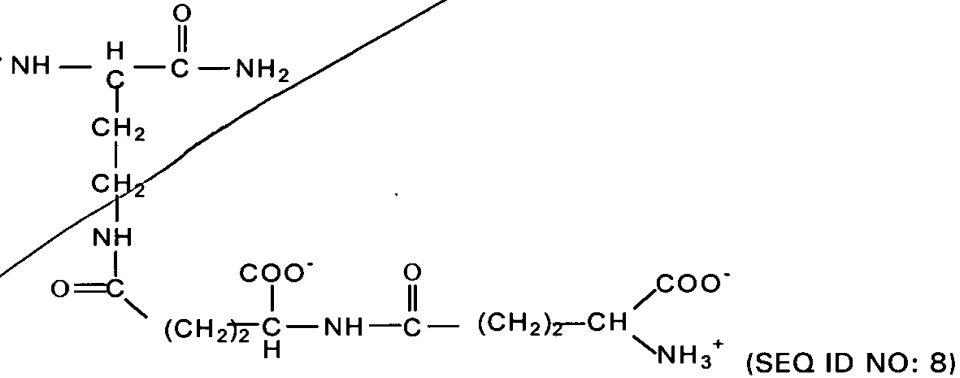
**[0044]** In sum, a set of particularly preferred procytotoxins have the following structures: (1) Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys(R)-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys(R)-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys(R) (SEQ ID NO: 1), and (2) Gly-Ile-Gly-Ala-Val-Leu-Lys(R)-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys(R)-Arg-Lys(R)-Arg-Gln-Gln (SEQ ID NO: 2), wherein R is independently selected from the group consisting of the ε-amino group of the adjacent lysine residue, [ε-γ]-Glu, [ε-γ]-Glu-[α-γ]-(Glu)<sub>1-3</sub>, [ε-α]-(Phe)<sub>1-3</sub>, [ε-α]-(Tyr)<sub>1-3</sub>, [ε-α]-(Trp)<sub>1-3</sub>, [ε-α]-(Lys)<sub>1-3</sub> and [ε-α]-(Arg)<sub>1-3</sub>, wherein [ε-γ] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate, [α-γ] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate, [ε-α] represents a peptide bond between the epsilon amino acid of lysine and the alpha carboxyl group of the indicated amino acid and the subscript indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds. With regard to the subscripted numbers, it is understood that larger numbers of amino acids are possible, *e.g.*, 4, 5, 6, *etc.*, but 1, 2, and 3 are anticipated to be optimal.

Please delete paragraph [0071], on pages 26 & 27, and replace it with the following paragraph:

*dses* ~~[0071] N-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-~~  
~~Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys-CONH<sub>2</sub> (SEQ ID NO: 3).~~

**Procytolytic Peptide:**

N-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp--



*B4* Example 2: Assay for the cytolytic activity of the pore-forming toxins

Please delete paragraph [0076], on page 28, and replace it with the following paragraph:

*B9* [0076] This example demonstrates that the inventive  $\gamma$ -glutamate-masked cytolytic peptides have specificity for cancer cells other than those expressing PSMA. This experiment, utilized a melittin analog having A  $[\epsilon\text{-}\gamma\text{-Glu-}\alpha\text{-}\gamma\text{-Glu}]$  at each of lysines 21 and 23:  $\text{NH}_2\text{-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys}([\epsilon\text{-}\gamma\text{-Glu-}\alpha\text{-}\gamma\text{-Glu}]\text{-Arg-Lys}([\epsilon\text{-}\gamma\text{-Glu-}\alpha\text{-}\gamma\text{-Glu}]\text{-Arg-Gln-Gln-COOH}$  (SEQ ID NO: 12). Two prostate tumors (PNCap and DU0145), two ovarian tumors (HeLa and SK-OV-3), one lung tumor (LLC1) and one melanoma (B16) were tested. Cultured cells were treated with 1, 10, 50 or 100  $\mu\text{M}$  peptide. Results, depicted in Figure 4, show strong lytic activity against all tumors.

Please insert the Sequence Listing filed concurrently herewith following the specification and before the claims, and renumber pages 1-6 of the Sequence Listing as pages 30-35 and renumber the claims and abstract concurrently beginning at page 36.